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Title of the Invention

DIPYRIDOXYL PHOSPHATE-NMRI-CONTRAST-AGENTS

SVCLV/C

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### Field of the Invention

This invention relates to novel compounds which form highly stable chelates with metal ions and which are useful as metal ion carriers for in vivo medical applications. In particular, this invention is directed to novel dipyridoxyl compounds which form highly stable chelates with polyvalent metal ions, the preparation of the compounds and chelates thereof with polyvalent ions and particularly paramagnetic ions, and the use of the paramagnetic chelates as contrast agents in nuclear magnetic resonance imagery (NMRI).

### Background of the Invention

Traditionally, chelates have been used to administer poorly soluble salts in medicine and as antidotes for detoxification in cases of heavy metal or heavy metal isotope poisoning. Chelates have also been used to deliver <sup>radioisotopes</sup> ~~radioisotopes~~ to areas of the body for imaging and radiation therapy. Most recently, chelates with paramagnetic contrast agents have been reported for use with NMRI.

Paramagnetic metal ions are frequently toxic in the concentrations required for use in NMRI, and introducing them into the body in the form of chelates renders them more physiologically acceptable. This requires that a chelate be able to hold the metal ion tightly in the chelate structure, that is, the formation constant for the chelate must be very large at physiological pH. The paramagnetic metal chelate must also be sufficiently soluble to permit administration of quantities required for imaging in reasonable volumes. Usual routes of administration are orally, intravenously and by enema.

The chelating agent must form a stable chelate with those paramagnetic metals which <sup>present</sup> ~~prevent~~ a hazard to the

body if released during use. Paramagnetic metals which are naturally present in the body are preferred. The chelate forming agent (ligand) must be capable of forming a chelate with a selected paramagnetic material without altering the metal's oxidation state or otherwise reducing its chemical stability.

Since the role of the paramagnetic metal in increasing contrast in NMRI imaging involves reducing the spin-lattice spin relaxation time  $T_1$  and the spin-spin relaxation time  $T_2$ , the chelate structure must hold the metal ion tightly while permitting contact of the metal ion with protons in water molecules.

This invention provides a novel, superior chelating agent and metal complexes therewith which meet the above objectives.

#### DESCRIPTION OF THE PRIOR ART

A summary of the history and state of the art of contrast agents for NMRI is presented by Valk, J. et al, BASIC PRINCIPLES OF NUCLEAR MAGNETIC RESONANCE IMAGING. New York: Elsevier, pp 109-114 (1985). The Valk et al publication also describes the imaging equipment and methods for NMRI, and the entire contents of the Valk et al publication are hereby incorporated by reference in <sup>their</sup> ~~its~~ entirety. Chelates with ethylenediaminetetraacetic acid (EDTA) and diethylaminetriaminepentaacetic acid (DTPA) are described. Toxicity problems were reduced by seeking less toxic metal ions such as iron and gadolinium in a complex of gadolinium-DTPA chelate-meglumine. Gadolinium, however, is not naturally present in the body and long term toxicity studies have not been completed. Paramagnetic materials listed in this publication include molecules with unpaired electrons: nitric oxide (NO); nitrogen dioxide

H  
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H  
(NO<sub>2</sub>); and molecular oxygen (O<sub>2</sub>). Also included are ions with unpaired electrons, that is ions from the "transition series". Listed ions include Mn<sup>2+</sup>, Mn<sup>3+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup>, Cr<sup>2+</sup>, Cu<sup>2+</sup>, the

5 lanthanide series including gadolinium and europium, and nitroxide stable free radicals (NSFR) such as pyrrolidine NSFR and piperidine NSFR. Toxicity problems are indicated to present a major problem with many paramagnetic materials.

10 Use of alkylenediamine chelates with a variety of paramagnetic ions are described in U.S. Patent 4,647,447. Ferrioxamine-paramagnetic contrast agents are described in U.S. Patent 4,637,929. Manganese(II) is listed as a suitable paramagnetic metal ion for use  
15 with polysaccharide derivatives of a variety of chelating compounds including EDTA, DTPA and aminoethyl diphosphonate in PCT application publication no. WO85/05554 (Application No. PCT/GB85/00234). Stable radioactive diagnostics agents containing <sup>99m</sup>Tc  
20 chelated with N-pyridoxal- $\alpha$ -aminoacids or a pyridoxal salt are disclosed in U.S. Patents 4,313,928, 4,440,739, and 4,489,053.

B  
H  
B  
B  
B  
Taliaferro, C. et al in "New multidentate ligands. 22. N,N'-dipyridoxyethylenediamine-N,N'-diacetic acid: a new chelating ligand for trivalent metal ions",  
25 Inorg.Chem. 23:1188-1192 (1984) describes development of N,N'-dipyridoxyethylenediamine-N,N'-diacetic acid (PLED) as a chelating compound for trivalent metal ions. Other chelating compounds described are the Fe(III) chelates  
30 of N,N'-ethylenebis-2-(o-hydroxyphenyl)glycine (EHPG) and N,N'-bis(1-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED). Properties of chelates of PLED, HBED, EHPG and EDTA with ions of copper, nickel, cobalt,

zinc, iron, indium and gallium are compared.

Investigation of the structure of PLED is reported by Taliaferro, C. et al, Inorg.Chem. 24:2408-2413 (1985).

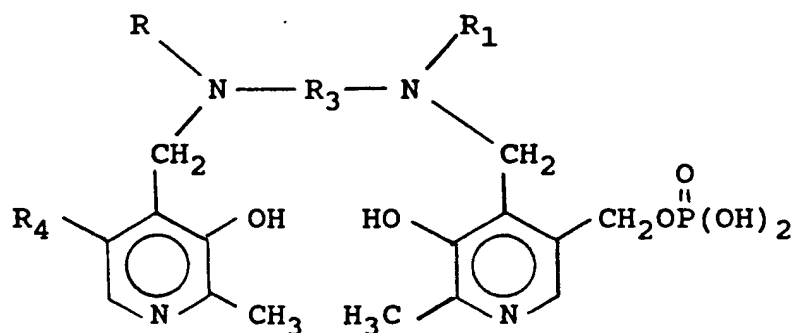
Green, M. et al, Int.J.Nucl.Med.Biol. 12(5):381-386 (1985) report their evaluation of PLED as a chelating ligand for the preparation of gallium and indium radiopharmaceuticals, and summarize properties of PLED chelates with Ga(III), In(III), and Fe(III).

Because the compounds of this invention have an aromatic hydroxy group, their value as  $\Delta$  chelating agents for manganese(II) ions would not be expected; such aromatic hydroxy groups would be expected to react with the manganese(II) ion as an oxidant in the usual way, oxidizing the manganese(II) ion to a higher valence. Frost, et al, J.Am.Chem.Soc. 80:530 (1958) report the formation of Mn(II) chelates of EHPG at low pH, but found that attempts to prepare stable manganese(II) complexes with EHPG at higher pH's (above pH 5) was futile as the manganese(II) ion was irreversibly oxidized. This oxidation occurred even under inert atmospheres, and the writers concluded that the oxidation occurred at the expense of the ligand or solvent. Anderegg, G. et al, Helv.Chim.Acta. 47:1067 (1964) found the high stability of the Fe(III) chelate of EHPG was due to the high affinity of the Fe(III) ion for the two phenolate groups present in the ionized ligand. L'Eplattenier, F. et al, (J.Am.Chem.Soc. 89:837 (1967) describes studies of HBED involving acid titrations of HBED in the presence of a variety of metal ions, including manganese(II). No manganese chelate was isolated, and the manganese products were not characterized. Based on subsequent work by Patch et al, Inorg.Chem. 21(8):2972-2977 (1982), it is clear that the

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B  
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manganese(II) ion was oxidized by the phenolic ligand during the titrations of L'Eplathenier et al. Patch et al prepared a Mn(III) complex by reacting Mn(II) salts with EHPG, and concluded the reaction involved the  
5 oxidation of the ligand in an irreversible reaction. The ability to maintain Mn(III) in the +3 oxidation state was said to be a unique characteristic of the EHPG molecule. U.S. Patent 3,632,637 describes phenolic chelating agents such as N,N'-di(o-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid and their use in  
10 chelating trivalent and tetravalent metals <sup>These agents</sup> which are usually stable in the presence of aromatic hydroxy groups. No use of a compound with an aromatic hydroxy group as a chelating agent for manganese(II) ions is  
15 disclosed in these references, confirming the general knowledge about the oxidizing properties of the aromatic hydroxy group on manganese compounds, in particular manganese(II) ions.

#### SUMMARY OF THE INVENTION

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P  
The novel chelate forming compounds of this invention are shown in Formula I.

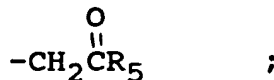


(I)

PS  
P  
T<sub>1</sub> 70X  
5  
P H  
T<sub>1</sub> 71X  
PS H  
PH B  
B  
B  
PH B  
T<sub>1</sub> 72X  
15  
PH  
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B  
25  
B14  
B  
30

wherein

R is hydrogen or

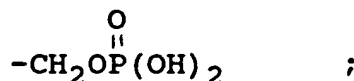


R<sub>1</sub> is hydrogen or



and one of R and R<sub>1</sub> is other than hydrogen;

10 R<sub>3</sub> is alkylene having from 1 to 8 carbons,  
1,2-cycloalkylene having from 5 to 8 carbons, or  
1,2-arylene having from 6 to 10 carbons, and  
R<sub>4</sub> is hydrogen, alkyl having from 1 to 6 carbons  
or



20 R<sub>5</sub> and R<sub>6</sub> are each, individually, hydroxy,  
alkoxy having from 1 to 18 carbons,  
hydroxy-substituted alkoxy having from 1 to 18  
carbons, amino or alkylamido having from 1 to 18  
carbons.

The phosphate group mono and diesters with mono and  
polyhydric alkanols having from 1 to 18 carbons, or  
25 alkylamino alcohols, each having from 1 to 18 carbons,  
and the salts of the above compounds are included within  
the scope of this invention.

Also included in this invention are the chelates of  
the compounds of Formula I and salts and esters thereof  
30 with metal ions, preferably paramagnetic metal ions  
having atomic numbers of from 21-29, 42, 44 and 58-70,  
and optimally manganese(II), and their use as imaging  
agents.

DRCLV/C  
P  
B  
3  
B

The novel intermediate compounds from which the compounds of Formula I are prepared are also included within the compounds of this invention.

Brief Description of the Drawing

5 Fig. 1 shows a structural formula of a species of N,N'-bis(pyridoxal-5-phosphate)ethylenediamine-N,N'-diacetic acid, showing the dissociation constants, pK's, as assigned to the protonation sites described in Example 11.

B  
DECLV/C  
P

10 Fig. 2 is a graph showing the relationship between dosage and relaxivity using the Mn(DPDP) compound of this invention based on the data shown in Example 9.

Detailed Description of the Invention

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B  
B H

15 The novel chelate forming compounds of this invention are shown in Formula I. The pharmaceutically acceptable water-soluble compatible salts of the compounds of Formula I and phosphate group esters of the compounds of Formula I with polyhydric alcohols, aliphatic alcohols, or alkylamino alcohols, each having  
20 from 1 to 18 carbons, and the chelates thereof are also included within the compounds of this invention.

B  
B H

25 In Formula I, R<sub>5</sub> and R<sub>6</sub> are preferably each individually hydroxy, alkoxy having from 1 to 8 carbons, ethylene glycol, glycerol, amino or alkylamido having from 1 to 8 carbons. Optimally, R<sub>5</sub> and R<sub>6</sub> are hydroxy and the salts thereof.

B  
B  
B

30 The term "alkyl" and "alkylene", as used herein, include both straight and branch-chained, saturated and unsaturated hydrocarbons. The term "1,2-cycloalkylene" includes both cis and trans cycloalkyl groups and alkyl substituted cycloalkylene groups bonded at the 1,2-positions to respective nitrogen atoms and alkyl substituted derivatives thereof having from 5 to 8



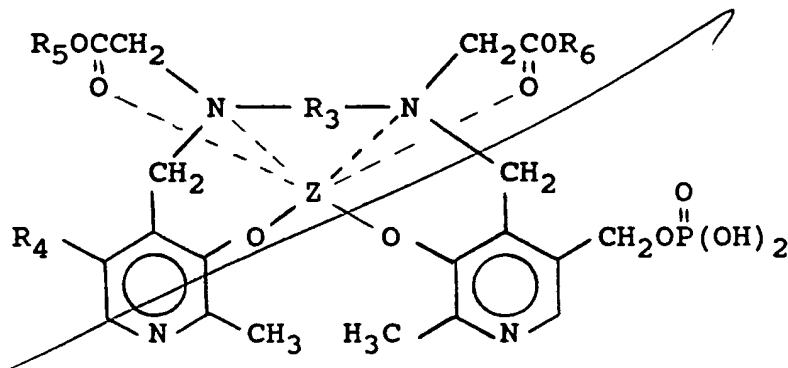
carbons. The term "1,2-arylene" includes phenyl and naphthyl groups bonded at the 1,2-positions to respective nitrogen atoms and alkyl substituted derivatives thereof, having from 6 to 10 carbons.

The compound, N,N'-bis-(pyridoxal-5-phosphate)-ethylenediamine-N,N'-diacetic acid or N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridyl-methyl)ethylenediamine-N,N'-diacetic acid, is referred to hereinafter as DPDP, and the Manganese(II) chelate is referred to hereinafter as Mn(DPDP).

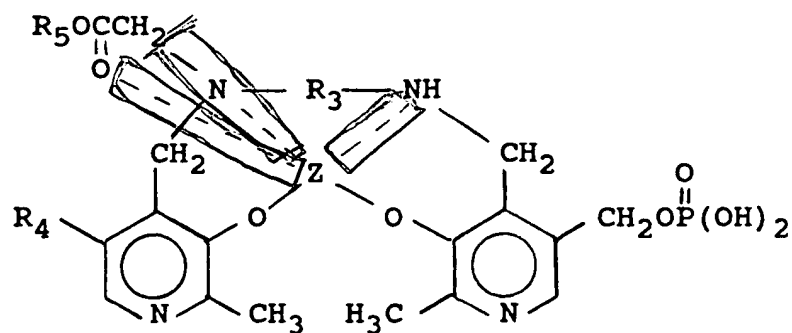
The dicarbonyl compound of Formula I, when R<sub>5</sub> and R<sub>6</sub> are hydroxy and R<sub>3</sub> is ethylene, is DPDP. DPDP has the dissociation constants for the protonation sites shown in Fig. 1. As described in Example 11, at pH of 3 and above, the ligand is anionic and possesses deprotonated metal binding sites, both important criteria for metal chelating agents.

#### CHELATES

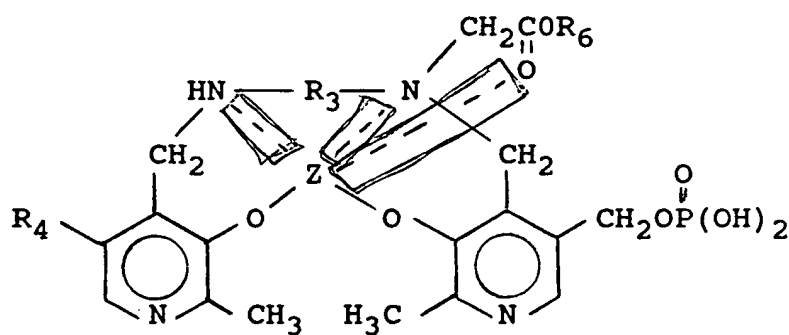
The chelates of this invention are chelates of the compounds of Formula I with metal ions. The chelates can be represented by Formulas II, III or IV.



(II)



(III)



(IV)

In Formulas II, III and IV, Z represents a metal ion and  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are the same as described with respect to the compounds of Formula I. The dotted lines in the figure represent the dative bonding between the oxygen and nitrogen atoms and the metal ion. One of the acetyl groups in Formula II is below the plane of the aromatic pyridine rings and the other acetyl group is above the plane of the aromatic pyridine rings, so the metal ion is tightly held within the interior of the chelate salt complex with the dicarboxy embodiments of this invention. Also included in the chelates of this invention are the pharmaceutically acceptable

water-soluble compatible salts, and carboxylic and phosphate group esters with hydroxy-substituted alkanols, alkanols, or alkylamino alcohols, each having from 1 to 18 carbons, of the compounds of Formulas II, III, <sup>and</sup> IV.

For use as a medium for NMRI analysis, the central ion of the complex chelate salt must be paramagnetic, and preferably is a divalent or trivalent ion of elements with an atomic number of 21 to 29, 42, 44 and 58 to 70. Suitable ions include chromium(III), manganese(II), iron(III), iron(II), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III), ytterbium(III). Gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III) are sometimes preferred because of their strong magnetic moments and chemical stability, but because they are not normally present in the body, their long term biological effects are unknown.

With the novel chelate forming compounds of Formula I, chelates of manganese(II) are preferred. Relatively few manganese(II) chelate compounds are known, and only a fraction of these have been characterized ~~including single crystal X-ray diffraction work~~. Most of the structurally characterized Mn(II) complexes have various mono and bidentate ligands coordinating to the metal center. The Mn(II) complexes of Formulas II, II and III, and the Mn(II) complexes with PLED and the corresponding 1,2-cycloalkylene and 1,2-arylene compounds described in our co-pending, <sup>U.S. Serial No. 47,584 filed May 8, 1987</sup> concurrently filed application titled MANGANESE(II) CHELATE CONTRAST AGENTS AND METHODS (attorney docket no. ~~145.0003~~) are the first Mn(II) complexes with a high

H  
H B B  
3 BH B  
B 3  
B  
B

3  
BH  
B  
B  
3  
B  
B

B  
B

12

DA BA  
U/L  
B

The elements of the above-listed atomic numbers which form the central ion or ions of the physiologically well tolerated chelate salt, must not be radioactive for the intended use of the diagnostic medium for X-ray diagnosis and NMRI. Radioactive metal ion chelates of the compounds of Formula I are described in our ~~co-pending, concurrently filed application~~ <sup>3206</sup> titled DIPYRIDOXYL PHOSPHATE RADIOACTIVE METAL CHELATES ~~(Attorney Docket No. 145.0004)~~.

10 For purposes of clarity, the chelates of this invention will be described hereinafter in terms of paramagnetic ions suitable for use in NMRI analysis. However, this is for purposes of clarity of explanation and not by way of limitation, and chelates of all of the  
15 above metal ions are included within the scope of this invention.

If not all of the active hydrogen atoms of the chelates are substituted by the central paramagnetic ion, the solubility of the chelate is increased if the  
20 remaining hydrogen atoms are substituted with physiologically biocompatible cations of inorganic and/or organic bases or amino acids. For example, the lithium ion, the potassium ion, the sodium ion and especially the calcium ion are suitable inorganic  
25 cations. Suitable cations of organic bases include, for example, ethanolamine, diethanolamine, morpholine, glucamine, N,N-dimethylglucamine, <sup>and</sup> N-methylglucamine.  
B Lysine, arginine or orithine are suitable as cations of amino acids, as generally are those of other basic  
30 naturally occurring acids.

B  
B The preferred calcium salts have calcium ion to chelating molecule mole ratios of from 0.05 to 1.0, the optimum mole ratios being with the range of from 0.1 to

0.5. At mole ratios of calcium ion to chelate molecule above 1.0, the chelate tends to become insoluble. The soluble calcium salts are most physiologically acceptable since they do not significantly disturb the concentration of free calcium ions in the patient's system.

The chelates according to this invention are formed from the chelate forming compounds of Formula I by conventional procedures known in the art. In general, these processes involve dissolving or suspending the metal oxide or metal salt (for example, nitrate, chloride or sulfate) of an element with an atomic number of 21 to 29, 42, 44 or 57 to 83 (for example, oxides or salts of  $Mn^{+2}$ ,  $Cr^{+3}$ ,  $Fe^{+2}$ ,  $Fe^{+3}$ ,  $Co^{+3}$ ,  $Ni^{+2}$ ,  $Cu^{+2}$ ,  $Pr^{+3}$ ,  $Nd^{+3}$ ,  $Sm^{+3}$ ,  $Yb^{+3}$ ,  $Gd^{+3}$ ,  $Tb^{+3}$ ,  $Dy^{+3}$ ,  $Ho^{+3}$ , or  $Er^{+3}$ ) in water or a lower alcohol such as methanol, ethanol or isopropanol. To this solution or suspension is added an equimolar amount of the chelating acid in water or a lower alcohol, and the mixture is stirred, if necessary, with heating moderately or to the boiling point, until the reaction is completed. If the chelate salt formed is insoluble in the solvent used, the reaction product is isolated by filtering. If it is soluble, the reaction product is isolated by evaporating the solvent to dryness, for example, by spray drying or lyophilizing.

If acid groups such as the phosphoric acid groups are still present in the resulting chelate, it is advantageous to convert the acidic chelate salt into a neutral chelate salt by reaction with inorganic and/or organic bases or amino acids, which form physiologically biocompatible cations, and to isolate them. This is often unavoidable since the dissociation of the chelate

salt is moved toward neutrality to such an extent by a shift in the pH value during the preparation that only in this way is the isolation of homogeneous products or at least their purification made possible. Production  
5 is advantageously performed with organic bases or basic amino acids. It can also be advantageous, however, to perform the neutralization by means of inorganic bases (hydroxides, carbonates or bicarbonates) of sodium, potassium or lithium.

10 To produce the neutral salts, enough of the desired base can be added to the acid chelate salts in an aqueous solution or suspension that the point of neutrality is reached. The resulting solution can then be concentrated to dryness in vacuo. It is often  
15 advantageous to precipitate the neutral salts by adding a solvent miscible with water, for example, lower alcohols (methyl, ethyl, isopropyl alcohols, etc.), lower ketones (acetone, etc.), polar ethers (tetrahydrofuran, 1,2-dimethoxyethane, etc.) and thus  
20 obtain crystals that isolate easily and purify well. It has been found particularly advantageous to add the desired bases to the reaction mixture even during chelating and thus eliminate a process stage. Other conventional purification procedures such as column  
25 chromatography can be used.

Since the chelate salts of Formulas II, III and IV contain a plurality of acid groups, it is possible to produce neutral mixed salts which contain both inorganic and organic physiologically biocompatible cations as  
30 counterions. This can be done, for example, by reacting the complexing acids in an aqueous suspension or solution with the oxide or salt of the element supplying the central ion or less than the full amount of an

organic base necessary for neutralization, e.g., half, isolating the chelate salt that is formed, purifying it, if desired, and then adding it to the amount of inorganic base necessary for complete neutralization.

5 The sequence of adding the bases can be reversed.

B  
B  
B  
B  
10 The carboxylic and phosphoric acid groups of the chelating agents can also be neutralized by esterification to prepare carboxylate and phosphate esters. Such esters can be prepared by conventional procedures known in the art, for example, from the corresponding alcohols. Suitable esters include, for example, esters of straight or branch-chained alkanol groups having from 1 to 18 carbons, mono and polyhydric alkyl amino alcohols having from 1 to 18 carbons and  
15 preferably from 1 to 6 carbons such as serinol or diethanolamine, and polyhydric alcohols having from 1 to 18 and preferably from 1 to 6 carbons such as ethylene glycol or glycerol.

20 The diagnostic media for administration is formed using physiologically acceptable media in a manner fully within the skill of the art. For example, the chelate salts, optionally with the addition of pharmaceutically acceptable excipients, are suspended or dissolved in an aqueous medium, and then the solution or suspension is  
25 sterilized. Suitable additives include, for example, physiologically biocompatible buffers (as, for example, tromethamine hydrochloride), slight additions of other chelating agents (as for example, diethylenetriamine-pentacetic acid) or, ~~if~~ optimally, calcium salts (for  
B  
30 example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate).

Alternatively, the diagnostic media according to this invention can be produced without isolating the



chelate salts. In this case, special care must be taken to perform the chelating so that the salts and salt solutions according to the invention are essentially free of unchelated, potentially toxic metal ions. This can be assured, for example, using color indicators such as xylenol orange to control titrations during the production process. A purification of the isolated salt chelate can also be employed as a final safety measure.

If suspensions of the chelate salts in water or physiological salt solutions are desired for oral administration, a small amount of soluble chelate salt

*Can be*  
is mixed with one or more of the inactive ingredients traditionally present in oral solutions *such as* and/or surfactants, *and the like* and/or aromatics for flavoring.

The most preferred mode for administering paramagnetic metal chelates as contrast agents for NMRI analysis is by intravenous administration. Intravenous solutions must be sterile, free from physiologically unacceptable agents, and should be isotonic or iso-osmotic to minimize irritation or other adverse effects upon administration. Suitable vehicles are aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, and other solutions such as are described in REMINGTON'S PHARMACEUTICAL SCIENCES. 15th Ed., Easton: Mack Publishing Co. pp 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV. 14th Ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. The solutions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used in parenteral

solutions, selecting excipients and other additives which are compatible with the chelates and which will not interfere with the manufacture, storage or use of the products.

5           The diagnostic media according to this invention can contain from 0.001 to 5.0 moles per liter and preferably from 0.1 to 0.5 moles per liter of the chelate salt.

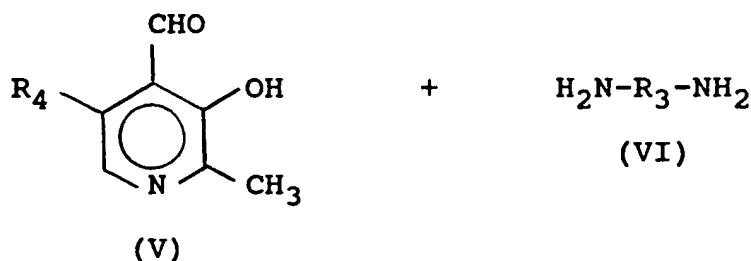
The chelates of this invention are administered to patients for imaging in amounts which are sufficient to yield the desired contrast. Generally, dosages of from 0.001 to 5.0 mmoles of contrast agent per kilogram of patient body weight are effective to achieve reduction of relaxivity rates. The preferred dosages for most NMRI applications are from 0.02 to 0.5 mmoles of contrast agent per kilogram of patient body weight.

Methods for applying the contrast agents to improve NMRI images, equipment and operating procedures are described by Valk, J. et al, supra. The contrast agents can be used orally and intravenously.

20 In a novel NMRI application, the contrast agents can  
be introduced into the cervix, uterus and fallopian  
tubes. NMR imaging can then be performed to detect  
causes of infertility such as obstructions or  
imperfections in the internal surface of the fallopian  
25 tubes which might interfere with the movement of the  
fertilized ovum.

## CHELATE FORMING COMPOUNDS

The compounds of Formula I can be formed by reacting the corresponding pyridoxal 5-phosphate (3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridinecarboxyaldehyde) 30 represented by Formula V with <sup>a</sup>an diamine represented by Formula VI.



PSH<sub>B</sub>

B

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In the compounds of Formula *V and VI*,  $\text{R}_3$  and  $\text{R}_4$  are as defined with respect to Formula I. Pyridoxyl 5-phosphate, pyridoxal, and the other compounds of Formula V, and the alkylenediamine, cycloalkylenediamine and arylene reactants of Formula VI are well known compounds readily available from commercial sources, and

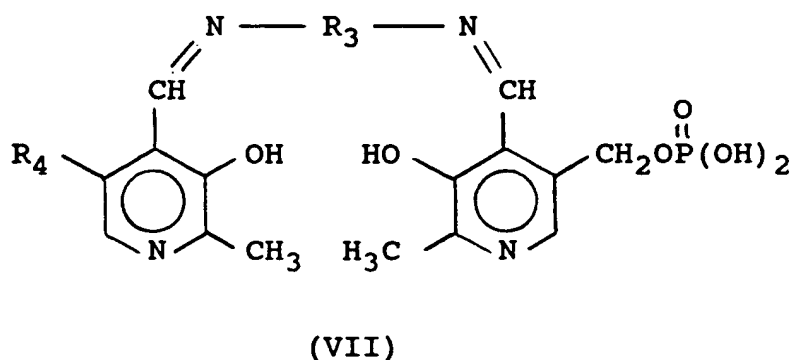
15 they can be readily synthesized by well known procedures fully within the skill of the art.

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The reaction of the amino groups of the compounds of Formula VI with the aldehyde group of the compounds of Formula V can be carried out in an alcohol such as methanol at a temperature within the range of from 0 to 60°C. The diimines formed are represented by Formula VII.



PSH

B

VII

In the compounds of Formula ~~V~~<sup>VII</sup>,  $R_3$  and  $R_4$  are the same as described with respect to the compounds of Formula I. For the manufacture of compounds wherein  $R_4$  is a phosphonomethyl group, i.e., the

5 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylideneaminoalkyleneiminomethyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphoric acids,

10 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylideneamino-1,2-cycloalkyleneiminomethyl)-2-hydroxy-3-methyl-5-pyridylmethyl phosphoric acids, and

15 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylideneamino-1,2-aryleneiminomethyl)-2-hydroxy-3-methyl-5-pyridylmethyl phosphoric acids of Formula ~~VI~~<sup>VII</sup>,  
a diamine of Formula VI is reacted with two molar equivalents of an aldehyde of Formula V having the 5-phosphonomethyl group such as pyridoxyl 5-phosphate. For preparation of compounds of Formula VII wherein  $R_4$  is other than a phosphonomethyl group, the diamine of Formula VI is first reacted with only one molar

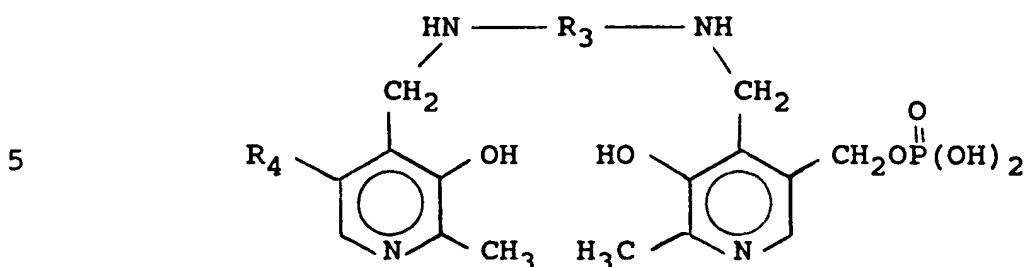
20 equivalent of an aldehyde of Formula V having the 5-phosphonomethyl group, and the mono-phosphonomethyl reaction product is reacted with one molar equivalent of a compound of Formula V having the desired  $R_4$  group, such as a 5-hydroxymethyl group, i.e, pyridoxal. The

25 reverse order of reaction can also be used. The reaction products of Formula VII are insoluble in the alcohol and can be isolated by filtration.

The compounds of Formula VII are then hydrogenated by conventional procedures using a palladium or platinum

30 catalyst to yield the diamines of Formula VIII.

T<sub>2</sub>10X



(VIII)

10 In the compounds of Formula VIII, *and*  $R_3$   $R_4$  are the same as described with respect to the compounds of Formula IV. The 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylaminoalkyleneaminomethyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphoric acids,  
 15 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylamino-1,2-cycloalkyleneaminomethyl)-2-hydroxy-3-methyl-5-pyridylmethyl phosphoric acids,  
 20 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)methylamino-1,2-cycloaryleneaminomethyl)-2-hydroxy-3-methyl-5-pyridylmethyl phosphoric acids, and the monophosponomethyl compounds of Formula VIII can be left in solution or isolated as crystalline solids.

25 The compounds of Formula I are prepared by reacting the diamines of Formula VIII with a haloacetic acid such as bromoacetic acid, the molar ratio of the bromoacetic acid to diamine determining whether one or both of the amines are conjugated with the acetic acid groups. The N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridyl-methyl)alkylenediamine-N,N'-diacetic acids,  
 30 N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridyl-methyl)-1,2-cycloalkylenediamine-N,N'-diacetic acids, N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridyl-methyl)-1,2-arylenediamine-N,N'-diacetic acids,

B  
B  
B  
B  
B  
5 N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridylmethyl)alkylenediamine-N-acetic acids, N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridylmethyl)-1,2-cycloalkylenediamine-N-acetic acids, and N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridylmethyl)-1,2-arylenediamine-N-acetic acids of Formula I are then isolated and purified by conventional procedures such as recrystallization or anion exchange chromatography.

B  
B  
B  
10 The carboxylic acid esters and amides can be formed by conventional procedures reacting the carboxylic acids with alkanols having from 1 to 18 carbons, hydroxy-substituted alkanols having from 1 to 18 carbons, ammonia, and alkylamines having from 1 to 18  
15 carbons.

This invention is further illustrated by the following specific but non-limiting examples. Temperatures are given in degrees centigrade and concentrations as weight percents unless otherwise  
20 specified. Procedures which are constructively reduced to practice herein are described in the present tense, and procedures which have been carried out in the laboratory are set forth in the past tense.

EXAMPLE 1

CL B  
CL B  
PB  
B  
B  
B  
B  
25 N,N'-bis(pyridoxal-5-phosphate)ethylenediimine  
A 265.2 gm (1 mole) quantity of pyridoxal-5-phosphate (Chemical Dynamics Corp., South Plainfield, NJ) was slurried in one liter of methanol, and 400 ml of 5 M NaOH was added. When the solution was homogeneous, 34.2  
30 ml of 1,2-diaminoethane (Aldrich Chem. Co.) was added rapidly with vigorous stirring. The imine product sodium N,N'-bis(pyridoxal-5-phosphate)ethylenediimine or

25

### N,N'-bis(pyridoxal-5-phosphate)alkyldiimines

30

**N,N'-bis(pyridoxal-5-phosphate)ethylenediamine**

145.0002

5

10

15

20

## 25

30



B  
B  
  
CLB  
CL  
PB  
B  
B  
B  
B  
B  
  
.  
  
B  
B  
  
B  
  
B  
  
B  
B  
B  
B  
B  
B  
B  
  
B  
B  
  
B  
B

## DPDP Synthesis

10

15

20

25

30

-25-

B  
B  
B  
CLB  
CL  
P B  
B  
B  
B  
B  
B14  
B  
B14  
B  
B  
B  
B  
B  
B14  
B  
B14

containing N,N'-bis-(pyridoxal-5-phosphate)ethylene-  
diamine-N,N'-diacetic acid or N,N'-bis(3-hydroxy-  
2-methyl-5-phosphonomethyl-4-pyridylmethyl)ethylene-  
diamine-N,N'-diacetic acid (DPDP).

5 EXAMPLE 6

DPDP Purification

The resinous solid obtained in Example 5 was  
dissolved in 600 ml of 88% formic acid, 1.5 liters of  
methanol followed by 2.2 liters of ethanol was added,  
10 and the mixture was cooled to 0°C for 2 hr. The solvent  
mixture was decanted from the resulting gum and  
discarded. The gum was dissolved in about 800 ml of  
deionized water which was then concentrated in vacuo to  
about 600-650 ml. Seed crystals were added, and the  
15 solution was allowed to stand at rm temp overnight. The  
product was isolated by filtration, washed with about  
400 ml of cold deionized water, 250 ml of ethanol, and  
then dried in vacuo to yield 65 gm of DPDP in 85-90%  
purity by HPLC. The filtrate and washings were  
20 retained, concentrated in vacuo to about 350 ml, and the  
solution refrigerated until column chromatographic  
purification of the second crop.

The 65 gms of product was then dissolved in 75 ml of  
88% formic acid containing 5 ml of deionized water with  
25 gentle heating to about 60°C. Cold deionized water was  
added to a total volume of one liter, and the solution  
was allowed to stand at 25°C for 16 hr to crystallize.  
The product was isolated by filtration, washed with 200  
ml cold deionized water, and dried in vacuo at 60°C to  
30 yield 55 gms of DPDP in 93-95% purity by HPLC. A second  
recrystallization, using the same procedure yields 50 gm  
of DPDP in 96-98% purity by HPLC, mp 174-180°C with  
decomposition. Analysis: (Calculated for

$C_{22}H_{32}N_4O_{14}P_2$ ) C, 41.38; H, 5.05; N, 8.77.  
 (Found) C, 40.70; H, 5.14; N, 8.61.  $^1H$  NMR ( $D_2O$ ,  
 400 MHz)  $\delta$  7.93 (s, pyr-H), 4.81 (d,  $CH_2OP$ ,  $J_{HP}$   
 = 6.3 Hz), 4.07 (s,  $NCH_2CH_2N$ ), 3.35 (s,  $CH_2COOH$ ),  
 2.83 (s,  $N-CH_2$ -pyr), 2.38 (s, pyr- $CH_3$ ).  $^{31}P$  NMR  
 ( $D_2O$ , 161 MHz)  $\delta$  -1.61 (s,  $CH_2OP$ ,  $H_3PO_4$   
 reference).

#### EXAMPLE 7

##### Sodium-Calcium Salt of Mn(DPDP)

10 A 4.16 gm (6.25 mmole) portion of DPDP from  
 Example 6 was dissolved in 15 ml of rigorously degassed  
 water by the addition of 1.0 gm (25 mmoles) of NaOH. A  
 1.25 gm (6.25 mmole) quantity of manganese dichloride  
 tetrahydrate was added, and the solution immediately  
 15 turned yellow. After stirring for 30 min, 0.25 gm (6.25  
 mmole) of solid NaOH was added to bring the pH up to  
 6.5. Then 0.15 gm (1.0 mmole) of calcium chloride was  
 added, and sufficient degassed water was added to bring  
 the volume of the solution to 25 ml. The clear yellow  
 20 solution was sterilized by being filtered through a 0.2  
 micron filter to yield the sodium-calcium salt of a  
 manganese chelate complex of N,N'-bis-(pyridoxal-5-phos-  
 phate)ethylenediamine-N,N'-diacetic acid or  
 N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-  
 25 4-pyridylmethyl)ethylenediamine-N,N'-diacetic acid.

#### EXAMPLE 8

##### Relaxivities with Mn(DPDP)

The relations of protons present in water and plasma  
 exposed to the chelate product of Example 7 was tested  
 30 by NMR for relaxities, in msec, at 10 MHz, 37°C. The  
 results are shown in Table I.

T<sub>2</sub> 80X

TABLE I

	Molar Conc.	<u>Relaxivities, msec.</u>			
		<u>T<sub>1</sub></u> <u>(Water)</u>	<u>T<sub>2</sub></u> <u>(Water)</u>	<u>T<sub>1</sub></u> <u>(Plasma)</u>	<u>T<sub>2</sub></u> <u>(Plasma)</u>
5	0.010	43	41	40	34
10	0.005	100	86	74	68
	0.0025	175		139	124
	0.00125	332		240	
	0.000625	639		398	
	0.000312	1083		624	
15	0.000156	1470		856	
	0.000078			995	
	0.000039			1103	

CLB  
CL

20

EXAMPLE 9

Organ Distribution of  
Mn(DPDP) in Rabbits

P

B  
B  
B

B

25

30

Each of four rabbits was injected intravenously with one of the following amounts of the solution obtained in Example 7: 0.01 mmoles/kg, 0.05 mmoles/kg, 0.10 mmoles/kg and 0.20 mmoles/kg. The rabbits were sacrificed 30 min post injection, and the proton relaxation values of selected body organs were measured with NMR, in vitro at 10 MHz. The relaxation rates found are shown in Table II.

T<sub>290X</sub>

TABLE II

5	<u>Relaxivities, msec.</u>										
	Normal			Observed							
	<u>Values</u>			<u>Values</u>							
	Dose (mmol/kg)			<u>0.01</u>		<u>0.05</u>		<u>0.10</u>		<u>0.20</u>	
	<u>Tissue</u>	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>
10	Brain	-	-	554	76	496	82	590	90	352	66
	Heart	605	70	-	-	353	54	300	51	205	47
	Lung	595	112	-	-	575	113	435	61	376	63
	Fat	171	154	-	-	200	139	183	115	192	-
15	Skel.										
	Musc.	423	47	-	-	494	47	425	31	232	31
	Renal										
	Cort.	338	85	298	65	210	57	188	55	143	53
	Renal										
20	Med.	672	149	502	99	223	57	209	48	127	60
	Liver	252	64	176	39	76	31	65	23	68	25
	Stom.	349	69	-	-	245	41	242	52	271	53
	Small										
	Int.	352	79	324	72	237	52	218	46	131	46
25	Large										
	Int.	349	77	283	64	365	83	290	57	256	74
	Urine	-	-	821	-	150	136	90	75	-	-
	Blood	900	-	844	-	613	-	506	-	411	-

30

p  
~  
T<sub>1</sub>300X  
PS  
B  
CLB  
p  
B  
B  
BH

The organ distribution data is plotted in Fig. 2 ,  
with the following symbols:

5      Liver   -l-      Heart   -h-      Cortex   -c-  
Medula   -m-      Urine   -u-      Blood   -b-

10      It shows rapid uptake of the Mn(DPDP) in the heart,  
liver and kidneys. The liver and kidneys are saturated  
with a dose of 0.10 mmole/kg while the heart continues  
15      to uptake Mn(DPDP) through the dose range studied. The  
complex of Mn(DPDP) may cross the intake-brain barrier  
as uptake by the brain was observed at higher doses. In  
cases where a defect is present in the blood-brain  
barrier (through disease or trauma), large amounts of  
15      the complex of Mn(DPDP) collect in the extravascular  
space and such defects were observed by NMRI  
tomography. The same defects are not observable without  
the use of Mn(DPDP) as a contrast agent.

EXAMPLE 10

20      Pharmacokinetics with Mn(DPDP)

Each of seven rabbits was injected intravenously  
with 0.01 mmol/kg of the solution obtained in  
Example 7. The rabbits were sacrificed at 0.25, 0.50,  
1.0, 2.0, 4, 6 and 24 hours post-injection, and the  
25      proton relaxation values of selected body organs were  
measured with NMR, in vitro, at 10 MHz. The T<sub>1</sub>  
relaxation rates are shown in Table III.

T<sub>1</sub> 310X

TABLE III

5

T<sub>1</sub> Relaxivities, msec

									T <sub>1</sub> Normal
	Time, hr	<u>0.25</u>	<u>0.50</u>	<u>1.0</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>24</u>	<u>Value</u>
10	<u>Tissue</u>								
	Liver	68	76	48	48	145	209	315	250 ±50
	Bile	160	46	25	21	<1	14	107	275 ±55
	Renal								
	cortex	202	191	229	192	210	239	328	338 ±60
15	Renal								
	medulla	192	231	236	310	340	375	563	672 ±100
	Heart	231	352	381	507	522	663	660	605 ±100

20

The pharmacokinetic data show rapid uptake and clearance of Mn(DPDP) in the liver, renal cortex, renal medulla and heart. The results indicate clearance of Mn(DPDP) through both the renal and hepatobiliary systems within 6-8 hours post-injection.

25

EXAMPLE 11

Potentiometric Titrations

The compound DPDP was studied potentiometrically from pH 11.2 to 2.0. Data sets were collected on a custom-built automatic potentiometric titration apparatus composed of a METROHM 655 DOSIMAT automatic buret, a FISHER ACCUMET pH meter with a CORNING calomel combination electrode, a custom-blown water jacketed

30

P  
B14  
CLB  
CL  
P  
B  
B

B  
B  
IB titration cell, a BRINKMAN LAUDA K-2/R constant temperature bath and a COMMODORE 64 computer. The BASIC computer program TITRATOR (Harris, W. et al, J.Am.Chem.Soc. 101:6534 (1979)) runs the apparatus.

5 Data analysis was performed on an IBM-AT computer using the least squares program, BETA (Harris, W. et al, supra), and the data analysis program, HANDNBAR (Harris, W. et al, supra). The titrants were standardized by phenolphthalein titration as follows: KOH was calibrated  
10 against potassium hydrogen phthalate (a primary standard), and HCl solutions were calibrated against the KOH standard. The Mn(II)Cl<sub>2</sub> solution was standardized with an EDTA titration using Erichrome Black T as the indicator. All solutions were made from distilled,  
15 deionized water that was further purified on a MILLI-Q cartridge system, degassed, and then kept under an atmosphere of argon which had been scrubbed for CO<sub>2</sub> and O<sub>2</sub>. Additions of EDTA and Mn(II) solutions were performed using calibrated GILMOT pipets. The electrode  
20 was calibrated in concentration units with degassed solutions of p[H+] = 2.291 and 1.078 at 0.1 M ionic strength.

B  
B  
25 The ligand proton titration was performed by adding 28.7 mg (0.045 mmoles) to 54.6 ml of a high pH aqueous solution. It was then titrated to low pH with 0.1009 N HCl.

B  
B  
30 The metal complex titration was performed by adding 152.2 mg (0.2383 mmoles) to 74.6 ml of a high pH aqueous solution. 2.07 ml of a 0.1152 M Mn(II) solution (0.2386 mmoles) were added. The complex was then titrated to low pH with 1.002 N HCl.

B  
The data obtained was analyzed using a model that consisted of eight one-proton steps. The first two



IB14  
3 B  
3  
B  
5 protonation equilibria were outside of the range of the titration window afforded by the concentration of titrant and were therefore estimated based on work by Martell and co-workers reported by Taliaferro, C. et al, Inorg.Chem. 24:2408-2413 (1985). Refinement of the remaining equilibria yielded constants with calculated e.s.d.'s (log K) of less than 0.02. Assignment of the protonation sites (pK's) was based on work by Martell and co-workers (Taliaferro, et al, supra), and is shown in Fig. 1.

CLB  
CLB  
PB  
B  
B  
POB  
POB  
POB  
POB  
POB  
POB  
POB  
At a pH of 3 and above, the ligand is anionic and possesses deprotonated metal binding sites, both important criteria for a metal chelating agent.

EXAMPLE 12

15 N,N'-bis-(pyridoxal-5-phosphate)-  
alkylenediamine-N,N'-diacetic acids

Repeating the procedure of Examples 5 and 6 but replacing the diamine of Example 3 with the products of Example 4 yields

20 N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-propylene)-  
N,N'-diacetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-propylene)-  
N,N'-diacetic acid,

25 N,N'-bis(pyridoxal-5-phosphate)-1,2-isopropylene-  
N,N'-diacetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-butylene)-  
N,N'-diacetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,4-(n-butylene)-  
N,N'-diacetic acid,

30 N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-butylene)-  
N,N'-diacetic acid, and

N,N'-bis(pyridoxal-5-phosphate)-1,2-(3-methylene)propyl-  
N,N'-diacetic acid.

CLB  
CL  
PB

H  
H  
H  
B

CLB  
CL  
PB

B  
H  
H  
H

POB

POB

POB

POB

EXAMPLE 13

DPDP Chelates

Repeating the procedure of Example 7 but replacing manganese dichloride tetrahydrate with equimolar amounts of the soluble chlorides of  $\text{Cr}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$ ,  $\text{Yb}^{+3}$ ,  $\text{Gd}^{+3}$ ,  $\text{Tb}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$ , or  $\text{Er}^{+3}$  yields the corresponding sodium salts of the respective metal ion chelates of N,N'-bis-(pyridoxal-5-phosphate)ethylene-diamine-N,N'-diacetic acid. The procedure can be repeated replacing the metal chloride salts with soluble nitrate or sulfate salts.

EXAMPLE 14

Other Chelates

Repeating the procedure of Example 7 but replacing N,N'-bis-(pyridoxal-5-phosphate)ethylenediamine-N,N'-diacetic acid with equimolar amounts of the chelate forming compounds produced in accordance with Example 10, and replacing manganese dichloride tetrahydrate with equimolar amounts of the soluble chlorides, carbonates or nitrates of  $\text{Cr}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$ ,  $\text{Yb}^{+3}$ ,  $\text{Gd}^{+3}$ ,  $\text{Tb}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$ , or  $\text{Er}^{+3}$  yields the sodium-calcium salts of the respective metal ion chelates of N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-propylene)-N,N'-diacetic acid, N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-propylene)-N,N'-diacetic acid, N,N'-bis(pyridoxal-5-phosphate)-1,2-isopropylene-N,N'-diacetic acid, N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-butylene)-N,N'-diacetic acid,

○  
○  
○  
○

N,N'-bis(pyridoxal-5-phosphate)-1,4-(n-butylene)-  
N,N'-diacetic acid,  
N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-butylene)-  
N,N'-diacetic acid, and  
5 N,N'-bis(pyridoxal-5-phosphate)-1,2-(3-methylene)propyl-  
N,N'-diacetic acid.

EXAMPLE 15

N,N'-bis(pyridoxal-5-phosphate)-  
trans-1,2-cyclohexylenediimine

10 A 26.5 gm quantity (0.1 mole) of pyridoxal-5-phos-  
phate was dissolved in 300 ml of methanol, and 38 ml of  
5 N NaOH was added. Then 5.71 gm (0.05 mole) of  
trans-1,2-diaminocyclohexane was added with stirring,  
and the volume of the solution was reduced to 200 ml in  
15 vacuo. After cooling to 0°C, the yellow imine was  
isolated by filtration, washed with diethyl ether, and  
dried in vacuo to yield 17 gm of sodium N,N'-bis(pyri-  
doxal-5-phosphate)-trans-1,2-cyclohexylenediimine or  
sodium 5-(N-(3-hydroxy-2-methyl-5-phosphonomethyl-  
20 4-pyridyl)methylideneamino-trans-1,2-cyclohexyleneimino-  
methyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphate (49%  
yield, melting point 200-205°C with decomposition).

EXAMPLE 16

Other N,N'-bis(pyridoxal-5-phosphate)-  
1,2-cyclo(alkylene or arylene)diimines

25 Repeating the procedure of Example 15 but replacing  
the trans-1,2-diaminocyclohexane with trans-1,2-diamino-  
cyclopentane, trans-1,2-diaminocycloheptane, trans-  
1,2-diaminocyclooctane, cis-1,2-diaminocyclohexane,  
trans-1,3-diaminocyclohexane, trans-1,4-diaminocyclo-  
30 hexane, o-aminoaniline and cis-1,4-diaminocyclohexane  
yields the corresponding

N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclopentylene-  
 diimine,  
 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cycloheptylene-  
 diimine,  
 5 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclooctylene-  
 diimine,  
 N,N'-bis(pyridoxal-5-phosphate)-1,2-phenylenediimine,  
 and  
 10 N,N'-bis(pyridoxal-5-phosphate)-cis-1,2-cyclohexylene-  
 diimine.

#### EXAMPLE 17

N,N'-bis(pyridoxal-5-phosphate)-  
 trans-1,2-cyclohexylenediamine

A 14 gm (0.02 mole) portion of the diimine product  
 15 of Example 15 was dissolved in 200 ml of 1:1 water:  
 methanol. The resulting solution was sparged with  
 argon, and 1.0 gm of 5% platinum on carbon was added.  
 The system was flushed with hydrogen. The hydrogen  
 pressure was increased to 50 psig for 16 hr at 25°C.  
 20 The reaction product was filtered through CELITE, and  
 the resulting solution of N,N'-bis(pyridoxal-5-phos-  
 phate)-trans-1,2-cyclohexyldiamine or sodium  
 5-(N-(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridyl)-  
 methylideneamino-trans-1,2-cyclohexyliminomethyl)-  
 25 2-hydroxy-3-methyl-5-pyridylmethylphosphate was  
 concentrated in vacuo to about 20 ml and cooled to 0°C  
 to induce crystallization. The product was isolated by  
 filtration, washed with cold H<sub>2</sub>O and dried in vacuo.  
<sup>1</sup>H NMR (D<sub>2</sub>O, 400 MHz) delta 7.45 (s, pyr-H), 4.53  
 30 (d, CH<sub>2</sub>OP, J<sub>HP</sub> = 4.9 Hz), 3.83 (dd, N-CH<sub>2</sub>-pyr),  
 2.72 (br s, cyclo-(CH<sub>2</sub>)<sub>4</sub>(CH)<sub>2</sub>(NH)<sub>2</sub>-), 1.88  
 (s, pyr-CH<sub>3</sub>), 1.83-1.08 (3 br s,  
 cyclo-(CH<sub>2</sub>)<sub>4</sub>(CH)<sub>2</sub>(NH)<sub>2</sub>-).

CLB  
CLB

EXAMPLE 18

N,N'-bis(pyridoxal-5-phosphate)-

1,2-cyclo(alkylene or arylene)diamines

Repeating the procedure of Example 17 but replacing  
5 the diimine product of Example 15 with the diimine  
products prepared in accordance with the procedure of  
Example 16 yields the corresponding diamines:

N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclopentylene-  
diamine,

10 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cycloheptylene-  
diamine,

N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclooctylene-  
diamine,

15 N,N'-bis(pyridoxal-5-phosphate)-1,2-phenylenediamine,  
and

N,N'-bis(pyridoxal-5-phosphate)-cis-1,2<sup>4</sup>-cyclohexylene-  
diamine.

EXAMPLE 19

N,N'-bis-(pyridoxal-5-phosphate)-trans-

20 1,2-cyclohexylenediamine-N,N'-diacetic acid

The diamine from Example 17 was charged to a one  
liter 3-neck flask, and the pH was adjusted to 11 with  
5 N NaOH. Then 5.6 gm (0.04 mole) of bromoacetic acid  
was dissolved in 10 ml of water and added dropwise to  
25 the stirred diamine solution while maintaining the pH at  
11. The reaction was warmed to 50°C and stirred for 16  
hr. 50 gm of weakly acidic cation exchange resin  
(AMBERLITE IRC-50) was added, and the pH dropped to  
6.7. The resin was removed by filtration, and 15 gm of  
30 cation exchange resin (DOWEX 50W-X8) was added. The pH  
dropped to 3.8.

The solution was filtered, and all of the solvent  
was evaporated from the filtrate to yield a foamy

solid. The solid was dissolved in 30 ml of 88% formic acid, and the product was precipitated by the addition of 150 ml of methanol followed by 150 ml of ethanol. The solvent mixture was decanted from the gummy solid and discarded. The solid was dissolved in a minimum amount of deionized water (about 100 ml), and the product was allowed to stand overnight at 25°C. The product was isolated by filtration, washed with 50 ml of cold water, 25 ml of ethanol and then dried in vacuo to yield the product. The compound was recrystallized by the same procedure to yield N,N'-bis-(pyridoxal-5-phosphate)-trans-1,2-cyclohexylenediamine-N,N'-diacetic acid or N,N'-bis(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridylmethyl)-trans-1,2-cyclohexylenediamine-N,N'-diacetic acid (DPCP) with a melting point (decomposition) of 221-226°C. <sup>1</sup>H NMR (D<sub>2</sub>O, 400 MHz) delta 7.53 (s, pyr-H), 4.58 (d, CH<sub>2</sub>OP, J<sub>HP</sub> = 5.9 Hz), 3.89 (dd, N-CH<sub>2</sub>-pyr), 3.31 (s, CH<sub>2</sub>COOH), 2.78 (br s, cyclo-(CH<sub>2</sub>)<sub>4</sub>(CH)<sub>2</sub>(NH)<sub>2</sub>-), 1.93 (s, pyr-CH<sub>3</sub>), 1.90-1.15 (3 br s, cyclo-(CH<sub>2</sub>)<sub>4</sub>(CH)<sub>2</sub>(NH)<sub>2</sub>-).

#### EXAMPLE 20

N,N'-bis-(pyridoxal-5-phosphate)-  
 cyclo(alkylene and arylene)diamine-  
 N,N'-diacetic acids

Repeating the procedure of Example 19 but replacing the diamine of Example 17 with the diamines of Example 18 yields the corresponding:  
 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclopentylene-diamine-N,N'-diacetic acid,  
 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cycloheptylene-diamine-N,N'-diacetic acid,

POB  
POB B5 3  
N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclooctylene-  
diamine-N,N'-diacetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-phenylenediamine-  
N,N'-diacetic acid, and

POB  
CLB  
CL  
PB  
B  
5 N,N'-bis(pyridoxal-5-phosphate)-cis-1,2<sup>4</sup>-cyclohexylene-  
diamine-N,N'-diacetic acid.

#### EXAMPLE 21

##### Chelates

Repeating the procedure of Example 7 but replacing  
10 N,N'-bis-(pyridoxal-5-phosphate)ethylenediamine-N,N'-di-  
acetic acid with equimolar amounts of the products of  
chelate forming compounds produced in accordance with  
Examples 19 and 20 and using equimolar amounts of the  
soluble chlorides, carbonates or nitrates of  $Mn^{+2}$ ,  
15  $Cr^{+3}$ ,  $Fe^{+2}$ ,  $Fe^{+3}$ ,  $Co^{+3}$ ,  $Ni^{+2}$ ,  $Cu^{+2}$ ,  $Pr^{+3}$ ,  
 $Nd^{+3}$ ,  $Sm^{+3}$ ,  $Yb^{+3}$ ,  $Gd^{+3}$ ,  $Tb^{+3}$ ,  $Dy^{+3}$ ,  $Ho^{+3}$ ,  
or  $Er^{+3}$  yields the sodium-calcium salts of the  
respective metal ion chelates of  
N,N'-bis(pyridoxal-5-phosphate)-trans-1,2<sup>cyclohexylene</sup>  
20 <sup>cyclopentylene</sup>-diamine-N,N'-diacetic acid,  
N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclopentylene-  
diamine-N,N'-diacetic acid,  
N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cycloheptylene-  
diamine-N,N'-diacetic acid,  
25 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclooctylene-  
diamine-N,N'-diacetic acid,  
N,N'-bis(pyridoxal-5-phosphate)-1,2-phenylenediamine-  
N,N'-diacetic acid, and  
30 N,N'-bis(pyridoxal-5-phosphate)-cis-1,2<sup>4</sup>-cyclohexylene-  
diamine-N,N'-diacetic acid.

CLB  
CLB

EXAMPLE 22

Pyridoxal-5-phosphate

(N-methylethanolamine)monoester

- 2.04 gm (0.01 mole) of pyridoxal hydrochloride is  
5 dissolved in 50 ml of dry THF containing 0.05 gm  
(0.02 mole) of sodium hydride with stirring. When gas  
evolution had ceased (about 15 min), 1.71 gm (0.01 mole)  
of benzyl bromide is added, and after stirring  
overnight, the solution is brought to dryness in vacuo.  
10 The sticky solid is suspended in 50 ml of dry methylene  
chloride and following addition of 3.0 gm (0.03 mole) of  
triethylamine, the slurry is cooled to 0°C. 1.38 gm  
(0.01 mole) of 2-chloro-3-methyl-1-oxa-3-aza-2-phospha-  
cyclopentane (prepared by the method of Jones, et al,  
15 J.Chem.Soc. Perkin trans I. p 199 (1985)) is added with  
vigorous stirring. The suspension is stirred for 1 hr  
at rm temp, and then 100 ml of water is added. The  
methylene chloride layer is separated, dried over  
MgSO<sub>4</sub>, and the solvent removed in vacuo. Addition of  
20 diethyl ether yields the intermediate product as a  
hygroscopic white solid (1.8 gm, 50% yield). The  
intermediate is oxidized with excess dinitrogen  
tetroxide in methylene chloride at -78°C, and then is  
treated with aqueous HCl in THF under reflux to give the  
25 (N-methylethanolamine)monoester of pyridoxal-5-phosphate  
in an overall yield of 40%.

EXAMPLE 23

N,N'-bis(pyridoxal-5-phosphate(N-methyl-  
ethanolamine)monoester)ethylenediimine

- 30 Repeating the procedure of Example 1 but replacing  
pyridoxal-5-phosphate with the product of Example 22  
yields N,N'-bis(pyridoxal-5-phosphate(N-methylethanol-  
amine)monoester)ethylenediimine or sodium



5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-4-pyridyl)-methylideneaminoethyleneiminomethyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphoric acid, N-methylethanolamine ester.

EXAMPLE 24

Other Monoester Diimines

Repeating the procedure of Example 23 with  
1,3-diamino-n-propane, 1,2-diamino-n-propane,  
1,2-diaminoisopropane, 1,2-diamino-n-butane,  
1,4-diamino-n-butane, 1,3-diamino-n-butane, *and*  
1,2-diamino-3-methylpropane yields the corresponding  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,3-(n-propylene)diimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-(n-propylene)diimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-isopropylenediimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-(n-butylene)diimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,4-(n-butylene)diimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,3-(n-butylene)diimine, *and*  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-(3-methyl)propylenediimine.

Repeating the procedure of Example 23 with  
trans-1,2-diaminocyclohexane, trans-1,2-diaminocyclo-  
pentane, trans-1,2-diaminocycloheptane, trans-1,2-di-  
aminocyclooctane, o-aminoaniline, and cis-1,2-diamino-  
cyclohexane, yields the corresponding  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-trans-1,2-cyclohexylenediimine,

POB  
B  
POB  
B  
POB  
B  
POB  
B  
POB  
B  
CLB  
CLB

N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-trans-1,2-cyclopentylenediimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-trans-1,2-cycloheptylenediimine,  
5 N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-trans-1,2-cyclooctylenediimine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-phenylenediimine, and  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
10 ester)-cis-1,2-cyclohexylenediimine.

EXAMPLE 25

N,N'-bis(pyridoxal-5-phosphate(N-methyl-  
ethanolamine)monoester)ethylenediamine

PB  
B  
B  
B  
B  
B  
CLB  
CL

Repeating the procedure of Example 3 but  
15 substituting the diimine product of Example 23 for the  
diimine product of Example 1 yields N,N'-bis(pyridoxal-  
5-phosphate(N-methyl-ethanolamine)monoester)ethylene-  
diamine or 5-(N-(3-hydroxy-2-methyl-5-phosponomethyl-  
4-pyridyl)methylaminoethyleneaminomethyl)-2-hydroxy-  
20 3-methyl-5-pyridylmethylphosphoric acid,  
N-methyl-ethanolamine ester.

EXAMPLE 26

Other Monoester Diamines

PB  
B  
B B  
POB B  
B  
POB  
B  
POB  
B

Repeating the procedure of Example 25 but  
25 substituting the <sup>diimine</sup> ~~diamine~~ products of Example 24 for the  
<sup>diimine</sup> ~~diamine~~ product of Example 23 yields  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,3-(n-propylene)diamine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
30 ester)-1,2-(n-propylene)diamine,  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-isopropylenediamine,

5  
10  
15  
20

DPDP-phosphate monoester

25

30

CLB  
CL

PB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

POB  
B

# EXAMPLE 28

## Other Diamine-N,N'-diacetic Acid Phosphate Monoesters

- Repeating the procedure of Example 27 but replacing
- 5 the products of Example 26 for the product of Example 25 yields
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ster)-1,3-(n-propylene)diamine-N,N'-diacetic acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- 10 ester)-1,2-(n-propylene)diamine-N,N'-diacetic acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-1,2-isopropylenediamine-N,N'-diacetic acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-1,2-(n-butylene)diamine-N,N'-diacetic acid salt,
- 15 N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-1,4-(n-butylene)diamine-N,N'-diacetic acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-1,3-(n-butylene)diamine-N,N'-diacetic acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- 20 ester)-1,2-(3-methyl)propylenediamine-N,N'-diacetic acid
- salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-trans-1,2-cyclohexylenediamine-N,N'-diacetic acid
- salt,
- 25 N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-trans-1,2-cyclopentylenediamine-N,N'-diacetic
- acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-trans-1,2-cycloheptylenediamine-N,N'-diacetic
- 30 acid salt,
- N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-
- ester)-trans-1,2-cyclooctylenediamine-N,N'-diacetic acid
- salt,

POB  
B  
POB  
B  
CLB  
CL  
PB  
B  
B  
B  
H  
H  
H  
H  
B  
CLB  
CL  
PB  
B  
H  
H  
H  
B

5 N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-1,2-phenylenediamine-N,N'-diacetic acid salt, and  
N,N'-bis(pyridoxal-5-phosphate(N-methylethanolamine)mono-  
ester)-cis-1,2-cyclohexylenediamine-N,N'-diacetic acid  
salt.

EXAMPLE 29

Chelates

Repeating the procedure of Example 7 but replacing  
N,N'-bis-(pyridoxal-5-phosphate)ethylenediamine-N,N'-di-  
10 acetic acid with an equimolar amount of the chelate  
forming compound produced in accordance with Example 27,  
yields the sodium-calcium Mn(II) chelate of  
N,N'-bis(pyridoxal-5-phosphate-(N-methyl-ethanolamine)-  
monoester)ethylenediamine-N,N'-diacetic acid.

15 Repeating this procedure, replacing manganese  
dichloride tetrahydrate with equimolar amounts of the  
soluble chlorides, carbonates or nitrates of  $\text{Cr}^{+3}$ ,  
 $\text{Fe}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  
 $\text{Sm}^{+3}$ ,  $\text{Yb}^{+3}$ ,  $\text{Gd}^{+3}$ ,  $\text{Tb}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$ , or  
20  $\text{Er}^{+3}$  yields the sodium-calcium salts of the respective  
metal ion chelates of N,N'-bis(pyridoxal-  
5-phosphate-(N-methyl-ethanolamine)monoester)ethylene-  
diamine-N,N'-diacetic acid.

EXAMPLE 30

Other Chelates

25 Repeating the procedures of Example 29 but replacing  
the products of Example 28 for the product of Example 27  
yields the  $\text{Mn}^{+2}$ ,  $\text{Cr}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  
 $\text{Ni}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$ ,  $\text{Yb}^{+3}$ ,  $\text{Gd}^{+3}$ ,  
30  $\text{Tb}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$ , or  $\text{Er}^{+3}$  ion chelates of the  
sodium-calcium salts of the diacetic acid chelating  
agents of Example 28.

CLB  
CL  
PB  
B  
B  
B  
B  
B

[illegible]

14

CLB  
CL  
PB  
B

EXAMPLE 32

Other (Mono)acetic Acids

Repeating the procedure of Example 31 with the products of Examples 4, 17 and 18 yields the corresponding

N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-propylene)diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-propylene)diamine-N-acetic acid,

10 N,N'-bis(pyridoxal-5-phosphate)-1,2-isopropylenediamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-(n-butylene)diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,4-(n-butylene)diamine-N-acetic acid,

15 N,N'-bis(pyridoxal-5-phosphate)-1,3-(n-butylene)diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-(3-methyl)propylene-diamine-N-acetic acid,

20 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclohexylene-diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclopentylene-diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cycloheptylene-diamine-N-acetic acid,

25 N,N'-bis(pyridoxal-5-phosphate)-trans-1,2-cyclooctylene-diamine-N-acetic acid,

N,N'-bis(pyridoxal-5-phosphate)-1,2-phenylenediamine-N-acetic acid, and

30 N,N'-bis(pyridoxal-5-phosphate)-cis-1,2-cyclohexylene-diamine-N-acetic acid.

CLB  
CL

PB  
B

H  
H  
H

B  
CLB  
CLB

PB

B  
B

B  
B  
B

B  
B

B  
B

B  
B  
B33  
B33

CLB  
CLB

PB  
B

### EXAMPLE 33

#### Other Chelates

Repeating the procedures of Example 29 but replacing the products of Example 32 for the product of Example 27 yields the  $Mn^{+2}$ ,  $Cr^{+3}$ ,  $Fe^{+2}$ ,  $Fe^{+3}$ ,  $Co^{+3}$ ,  $Ni^{+2}$ ,  $Cu^{+2}$ ,  $Pr^{+3}$ ,  $Nd^{+3}$ ,  $Sm^{+3}$ ,  $Yb^{+3}$ ,  $Gd^{+3}$ ,  $Tb^{+3}$ ,  $Dy^{+3}$ ,  $Ho^{+3}$ , or  $Er^{+3}$  ion chelates of the sodium-calcium salts of the monoacetic acid chelating agents of Example 32.

### EXAMPLE 34

#### N-pyridoxal-N'-(pyridoxal-5-phosphate)- ethylenediimine

A 25 gm (0.123 mole) quantity of pyridoxal hydrochloride is slurried in 100 ml of methanol, and 4.88 gm (0.123 mole) of NaOH is added. When the solution is homogeneous, it is added dropwise to 7.5 gm of 1,2-diaminoethane in 100 ml of methanol with stirring. After 60 min, a methanol solution containing 32.7 gm (0.123 mole) of pyridoxal-5-phosphate and 4.88 gm (0.123 mole) of NaOH is added with vigorous stirring. The unsymmetrical imine product, 5-(N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-methylideneaminoethyleneiminomethyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphoric acid or N-pyridoxal-N'-(pyridoxal-5-phosphate)-ethylenediimine, is stirred for 1 hr, and the product is isolated by filtration. The diimine is washed with methanol (2 x 50 ml) and diethyl ether (2 x 50 ml), and dried in vacuo.

### EXAMPLE 35

#### N-pyridoxal-N'-(pyridoxal-5-phosphate)- ethylenediamine

Repeating the procedure of Example 3 but substituting the diimine product of Example 34 for the



diimine product of Example 1 yields the corresponding diamine product,  
5-(N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-methylaminoethyleneaminomethyl)-2-hydroxy-3-methyl-5-pyridylmethylphosphoric acid or N-pyridoxal-N'-(pyridoxal-5-phosphate)-ethylenediamine.

EXAMPLE 36

DPMP Synthesis

Repeating the procedures of Example 5 and 6 with the product of Example 35 yields the corresponding N-pyridoxal-N'-(pyridoxal-5-phosphate)ethylenediamine-N,N'-diacetic acid or N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridylmethyl)-N'-(3-hydroxy-2-methyl-5-phosphonomethyl-4-pyridylmethyl)ethylenediamine-N,N'-diacetic acid (DPMP).

EXAMPLE 37

Other Chelates

Repeating the procedures of Example 29 but replacing the product of Example 36 for the product of Example 27 yields the  $Mn^{+2}$ ,  $Cr^{+3}$ ,  $Fe^{+2}$ ,  $Fe^{+3}$ ,  $Co^{+3}$ ,  $Ni^{+2}$ ,  $Cu^{+2}$ ,  $Pr^{+3}$ ,  $Nd^{+3}$ ,  $Sm^{+3}$ ,  $Yb^{+3}$ ,  $Gd^{+3}$ ,  $Tb^{+3}$ ,  $Dy^{+3}$ ,  $Ho^{+3}$ , or  $Er^{+3}$  ion chelates of the sodium-calcium salts of N-pyridoxal-N'-(pyridoxal-5-phosphate)ethylenediamine-N,N'-diacetic acid.